Acta Med. Nagasaki 36 : 73−84

Parental Origin and Mechanism of Formation of *de novo* Chromosome Abnormalities : 25 Cases of Numerical and Structural Abnormalities Determined by Restriction Fragment Length Polymorphisms

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Received for publication, March 27, 1991

ABSTRACT : Parental origin and mechanism of formation of *de novo* numerical and structural chromosome abnormalities were studied in 25 cases using RFLPs as genetic markers. In 8 of the 10 (5 autosomal and 5 X-chromosomal) numerical abnormalities studied, the origin and the mechanism of formation were ascertained. Of five 21-trisomics, two resulted from a maternal second meiotic nondisjunction, one (a 46/47,+21 mosaic) from mitotic nondisjunction of a paternally-derived chromosome 21, and the remaining two were uninformative. The origin and the mechanism of formation of the additional X chromosomes in the five patients with poly-X chromosomes (a case of XXXXX and four of XXXXY) studied were identical. They all arose through three nondisjunctions at maternal meiosis : once at the first meiosis and simultaneously twice at the second meiosis. These observations indicate that the parental origin of numerical abnormalities is not different between autosomes and X chromosome, the maternal origin being predominant.

Of the 15 structural abnormalities studied, the origin was ascertained in 11. An interstitial deletion of chromosome 15 [del(15)(q11.1q12)] in 2 of 5 cases arose at paternal meiosis. A 15q15q translocation in one of 2 cases resulted from centric misdivision of a maternal chromosome 15 followed by duplication of its long-arm, and thus the translocated chromosome is in the condition of "maternal uniparental disomy". A case of partial monosomy 21 (monosomy for 21pter-q21.3) resulted from a translocation between paternal chromosomes 2 and 21. The origin of X-chromosomal structural abnormalities in 3 cases were paternal and that in the other 4 cases maternal. Partial X-chromosome duplication [dup(Xp)] in one patient arose through an unequal sister chromatid exchange in the paternal X chromosome, partial deletion [del(Xp)] in one arose at the paternal meiosis, isochromosome X [i(Xq)] in three resulted from centric fission followed by duplication of Xq in a maternal X chromosome, isodicentric chromosome X [inv dup(Xq)] in one arose through an unequal exchange between sister chromatids in a maternal X chromosome, and ring chromosome X [r(X)] in the other case arose at maternal meiosis. These results on the structural abnormalities suggest that the *de novo* abnormalities due to events involving centromere disruption arise predominantly during oogenesis, while those due to simple breakage-reunion events occur preferentially during spermatogenesis.

INTRODUCTION

Most human *de novo* constitutional chromosome abnormalities occur during parental gametogenesis. The parental origin and mechanism of formation of the abnormalities, i.e., in which parent and in which meiosis (the first or the second meiotic division) the abnormalities occur, have been tried to be traced using chromosomal heteromorphisms as genetic markers. The origin was successfully ascertained in the trisomies for acrocentric chromosomes[5, 8, 15, 17, 18], in trisomy 16[14], and in polyploidy[9]. Also, with the same technique, the origin of several structural chromosome abnormalities has been ascertained[2]. However, this kind of study is restricted and applicable only to the chromosomes on which heteromorphisms exist.

Recently developed molecular genetics technique can detect DNA polymorphisms, such as restriction fragment lenght polymorphisms (RFLPs). There exist a number of RFLPs that are diversely distributed in the genome, and can be detected one in every several hundred base pairs of DNA. Theoretically, using RFLPs as markers, the origin of every chromosome abnormality can be ascertained. Thus, attempts with this technique were made to trace the origin of chromosome abnormalities, e.g,. partial monosomy 15[10, 11, 16], trisomy 18[12, 13], trisomy 21[20], monosomy X[6], and poly-X chromosomes[21].

The purpose of the present study is to ascertain the parental origin and the mechanism of formation of both numerical and structural abnomalities of autosomes and sex chromosomes.

MATERIALS AND METHODS

Subjects studied included a total of 25 patients with either autosomal or X chromosomal abnormalities, or with either numerical or structural abnormalities (**Table 1**): five patients with trisomy 21 [four standard 21-trisomics (patients 1-4) and one normal/trisomy 21 mosaic (patient 5)], one with pentasomy X (patient 6), four with XXXXY (patients 7-10), 5 with partial monosomy 15 (patients 11-15), two with 15q15q transocation (patients 16-17), one with partial monosomy 21 (patients 18), one with partial trisomy of Xp (patient 19), one with partial monosomy Xp (patient 20), three with isochromosome Xq (patient 21-23), one with isodicentric Xq (patient 24), and one with ring X-chromosome (patient 25). All of these were *de novo* unbalanced chromosome abnormalities (**Table 1**).

Genomic DNA of the patients and their respective parents was extracted from their peripheral blood leukocytes and/or Ebstein-Barr virus-transformed lymphoblastoid cell lines. The DNA was digested with endonucleases of interest (**Table 2**), electrophoresed on 0.8% agarose gel, denatured, and then Southern blotted onto nylon membranes with the standard methods. The DNA on the membrane was hybridized with ^{32}P-labeled DNA probes, and autoradiography was performed. Twenty-nine different cloned DNA segments were used in this study (**Table 2**). The copy number of each polymorphic DNA fragment in the patients and their parents was estimated by comparing the density for the fragment on the autoradiogram with that for an internal control DNA (pPA1 localized at 18q11.1-q12.1 and P20.36 localized at 11pter-p15.4), or by calculating a density ratio (R1) of one polymorphic autoradiographic band to the other, comparing with an average ratio (R2) among at least five normal individuals, *i.e.*, R1/R2.

RESULTS

Numerical abnormalities: The parental origin and the mechanism of formation of the numerical abnormalities could successfully be ascertained in 8 of the 10 patients studied (**Table 7**).

Trisomy 21: The genotypes of members of families 1-5 estimated by the RFLP study are shown in **Table 3**. The genotype of patient 1 for D21S110(p21-4U)/*Msp*I fragments was 18kb/18kb/7.0kb, that of father 7.0kb/7.0kb and that of mother 18kb/7.0kb (**Table 3, Fig. 1**). This indicated that two of three chromosomes 21 in this patient came in duplicate from her mother, *i.e.*, the origin of the additional chromosome 21

Table 1. Cytogenetic data on 25 patients studied

Patient number	Clinical diagnosis	Karyotype	Remarks
1	DS	47, XX, +21	trisomy 21
2	DS	47, XY, +21	trisomy 21
3	DS	47, XY, +21	trisomy 21
4	DS	47, XY, +21	trisomy 21
5	TAM	mos 46, XY/47, XY, +21	mosaic trisomy 21
6	MC	49, XXXXX	penta X
7	MC	49, XXXXY	three additional X
8	MC	49, XXXXY	three additional X
9	MC	49, XXXXY	three additional X
10	MC	mos 48, XXXY(10%)/49, XXXXY	mosaic poly-X
11	PWS	46, XY, del(15) (q11.1q12)	partial monosomy 15
12	PWS	46, XY, del(15) (q11.1q12)	partial monosomy 15
13	PWS	46, XY, del(15) (q11.1q12)	partial monosomy 15
14	PWS	46, XY, del(15) (q11.1q12)	partial monosomy 15
15	PWS	46, XY, del(15) (q11.1q12)	partial monosomy 15
16	PWS	45, XX, t(15 ; 15) (p11.1q11)	Robertsonian translocation
17	PWS	45, XY, t(15 ; 15) (p11.1q11)	Robertsonian translocation
18	MC	45, XY, -2, -21. +der(2) t(2 ; 21) (q37.3 ; q21.3)	partial monosomy 21
19	MC	46, X, dup(X) (p21)	partial trisomy X
20	TS	46, X, del(X) (p11)	partial monosomy X
21	TS	46, X, i(Xq)	partial monosomy Xp and partial trisomy Xq
22	TS	mos 45, X(66%)/46, X, i(Xq)	partial monosomy Xp and partial trisomy Xq
23	TS	mos 45, X(52%)/46, X, i(Xq)	partial monosomy Xp and partial trisomy Xq
24	PA	45, X, i dic(X) (q22 ; q22)	partial monosomy Xq and partial monosomy Xp
25	TS	mos 45, X(90%)/46, X, r(X) (p22.2 ; q26)	partial monosomies Xp and Xq

DS, Down syndrome ; TAM, transient abnormal myelopoiesis ; PWS, Prader-Willi syndrome ; MC, malformed child ; TS, Turner syndrome ; PA, primary amenorrhea. Percent values in parentheses in the karyotypes are the proportion of the former cell lines.

in the patient resulted either from a maternal second meiotic nondisjunction or from an early mitotic nondisjunction of maternally derived chromosome 21. On the other hand, the D21S82 (Fr8-77)/*Bam*HI genotype of the patient was 4.3kb/4.3kb/4.0kb, while that of the mother was 4.3kb/4.0kb, indicating the occurrence of meiotic crossing-over at a site proximal to the D21S82 locus (**Fig. 1**). This observation ruled out the possibility of the mitotic nondisjunction, retaining only the maternal second meiotic error. Similar RFLP segregations were observed in family 3 (**Table 3**), indicating that the parental origin and mechanism of formation of the trisomy 21 in patient 3 were identical to those in patient 1 (**Table 7**). The 46/47,+21 mosaic case (Patient 5) resulted from a mitotic nondisjunction of a paternally-derived chromosome 21. Families 2 and 4 were uninformative. RFLP studies just excluded the possibility of both paternal first and maternal second meiotic nondisjunctions in the former and that of paternal first meiotic nondisjunction in the latter (**Table 7**).

Poly-X chromosomes: In family 6, analysis with the probe/enzyme combination of DXS164 (pERT87-1)/*Xmn*I showed that the father was

Table 2. DNA Probes and endonucleases used in the present study

Chromosome	Map location	Probe	Gene name	Endonuclease	Polymorphic fragmment (Kb)
15	q11.2	pML34	D15S9	ScaI	6.5/6.3
	q11.2	pIR4-3R	D15S11	RsaI	1.2/1.0
				StyIa	3.4/2.4/1.9/0.9
	q11.2	pTD3-21	D15S10	TaqI	9.0/8.2
				EcoRy	23.0/7.0
				AIuIb	1.5/0.86+0.64
	q11.2	pIR39	D15S18	SacI	14/8.5+5.5
				BglII	9.0/8.5+0.5
21	q11.1	pPW511-1H	D21S52	HindIII	3.0/2.7+0.3
	q11.1	pPW552-3H	D21S59	TaqI	2.7/2.0
	q11.2 or q21.2	p21-4U	D21S110	MspI	18.0/7.0
	q11.2-q21	pPW228C	D21S1	MspI	7.8/4.6
	q11.2-q21	pPW236B	D21S11	EcoRI	2.9/1.9
	q11.2-q21.3	Fr8-77	D21S82	BamHI[c]	4.5/4.3/4.0
	q21	pPW245D	D21S8	HindIII	3.2/2.7
	q21	pPW513-5H	D21S54	MspI	1.8/1.1+0.7
	q21	pPW524-5P	D21S58	PstI	2.9/2.1+0.8
	q21.2	FB68L	APP	EcoRI	8.7/8.3
	q21.2	pGSE9	D21S16	XbaI	7.3/6.4
	q21.2-qter	pGSH8	D21S17	BglII	18.5/12.3
	q22.3	pPW231F	D21S3	TaqI	4.5/4.0
	q22.3	pGSE8	D21S15	MspI	4.1/3.4+0.7
	q22.3-qter	pGSB3	D21S19	PstI	3.6/2.2 ; 1.65/1.5
X	p22.3-22.2	L782	DXS85	EcoRI	14.0/7.0
	p22.1	p99-6	DXS41	PstI	22.0/13.0
	p21.3-p21.1	cDMD1a	DMD	PvuII[d]	20.0/5.8 ; 15.0/8.0
	p21.2	pERT87-1	DXS164	XmnI	8.7/7.5
		pERT87-15		BamHI	9.4/7.1+2.3
				TaqI	3.3/3.1
				XmnI	2.8/1.6+1.2
	p21.2	p20	DXS269	EcoRV	6.8/3.5 ; 2.1/1.8
	p21.2	J-Bir	DXS270	BamHI[e]	21.0/16.0+5.0
	p21.1	754-11	DXS84	EcoRI	4.2/2.4
	p11.4-p11.3	L1.28	DXS7	TaqI	12.0/9.0
	q28	DX13	DXS15	BglII	5.8/2.8

Data are from Human Gene Mapping 10 (1989)[7].
[a]Hamabe *et al.* (1990)[4] [b]Kamei *et al.* (1987)[10] [c]Abe *et al.* (1990)[1] [d]Deng *et al.* (1990)[3]
[e]Deng, unpublished data

a hemizygote for a 7.5kb allele and the mother a homozygote for a 8.7kb allele. The pentasomy X patient (patient 6) seemed heterozygote for both alleles, but densitometric analysis revealed that she had four copies of the 8.7kb allele and one copy of the 7.5kb allele (**Table 4, Fig. 2a**). Another combination, DXS269(p20)/*Msp*I, detected two pairs of polymorphic fragments (6.8kb/3.5kb, and 2.1kb/1.8kb) in the members of this family, and showed that the father was a hemizygote both for the 3.5kb and for the 2.1kb alleles and the mother a 6.8kb/3.5kb heterozygote and a 1.8kb/1.8kb homozygote. The patient seemed heterozygous both for the 6.8kb/3.5kb and for the 2.1kb/1.8kb fragment, and densitometry revealed two copies of the 6.8kb, three copies of the 3.5kb, one copy of the 2.1kb and four copies of the 1.8kb alleles (**Table 4, Fig 2b**). When the transmission of the three RFLPs above are combined, it was deduced that four of five X chromosomes in the patient had come from her mother, and two each of the four were derived from each homologue of the mother. This indicated that four of the five X

Table 3. Results of RFLP studies on the origin of trisomy 21 and monosomy 21

Family number	Family member	Genotypes for polymorphic alleles (Kb) at loci						
		D21S52	D21S110	D21S1	D21S11	D21S82	D21S17	D21S15
1	F		7/7	7.8/4.6	2.9/1.9	4.5/4.5		3.4/3.4
	M	3.0/3.0	18/7	7.8/7.8	2.9/1.9	4.5/4.3	18.5/18.5	4.1/3.4
	P	3.0/3.0/3.0	18/18/7	7.8/7.8/4.6	2.9/2.9/1.9	4.5/4.5/4.3[a]	18.5/18.5/18.5	4.1/3.4/3.4[a]
2	F	2.7/2.7	18/7	7.8/4.6	2.9/1.9		18.5/12.3	4.1/3.4
	M	3.0/2.7	7/7	4.6/4.6	2.9/2.9		18.5/18.5	3.4/3.4
	P	3.0/2.7/2.7	7/7/7	4.6/4.6/4.6	2.9/2.9/2.9		18.5/18.5/18.5	3.4/3.4/3.4
3	F	2.7/2.7	18/7	7.8/4.6	2.9/2.9	4.3/4.3	18.5/12.3	3.4/3.4
	M	2.7/2.7	18/18	4.6/4.6	2.9/2.9	4.5/4.3	18.5/12.3	4.1/3.4
	P	2.7/2.7/2.7	18/18/18	4.6/4.6/4.6	2.9/2.9/2.9	4.5/4.5/4.3	18.5/18.5/18.5	4.1/3.4/3.4[a]
4	F	3.0/2.7	18/7	4.6/4.6	2.9/1.9	4.5/4.5	18.5/12.3	4.1/3.4
	M	2.7/2.7	7/7	4.6/4.6	2.9/1.9	4.5/4.5	18.5/18.5	3.4/3.4
	P	2.7/2.7/2.7	7/7/7	4.6/4.6/4.6	2.9/2.9/1.9	4.5/4.5/4.5	18.5/18.5/18.5	4.1/3.4/3.4
5	F	2.7/2.7	7/7	7.8/7.8	1.9/1.9	4.5/4.3		4.1/4.1
	M		7/7		2.9/1.9			4.1/3.4
	P	2.7/2.7/2.7	7/7/7	7.8/7.8/4.6	2.9/1.9/1.9	4.5/4.3/4.3		4.1/4.1/3.4
18	F		7/7			4.0/4.0		
	M		7/7			4.3/4.3		
	P		7/-			4.3/-		

Underlined genotypes are informative.
[a]Genotypes inconsistent with those at proximal locus, indicating a crossing-over between the two loci.
F, M, P: father, mother, and patient

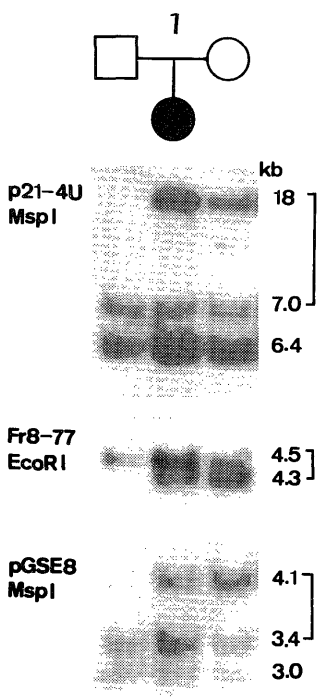

Fig. 1. Segregation of RFLPs at loci D21S110 (p21-4U), D21S82(Fr8-77) and D21S15 (pGSE8) in family 1 with trisomy 21.

chromosomes were due to three nondisjunctions at the maternal meiosis, once at the first meiosis and simultaneously twice at the second meiosis (**Table 7**).

The origin and the mechanism of formation of the additional X chromosomes in the other four cases (patients 7-10) of poly-X chromosome were identical to those in patient 6, although the RELPs leading to the conclusions were different in different patients (**Tables 4 and 7, Fig. 2c-k**).

Structural abnormalities: Of the 15 cases of structural abnormalities studied, the origin and the mechanism of formation were ascertained in 11 (**Table 7**).

Partial monosomy 15: The origin was ascertained in 2 (partients 11 and 12) of the 5 patients examined (**Tables 5 and 7**). In family 11, D15S10(pTD3-21)/*Taq*I-RFLP and densitometric analysis revealed that the father was a 9.0kb/9.0kb homozygote, the mother a 9.0kb/8.2kb homozygote, and the patient a 8.2kb hemizygote (**Table 5, Fig. 3a**), indicating that the del(15q) in this patient originated in the father (**Table**

Table 4. Results of RFLP studies on poly-X chromosomes

Family number	Family member	Genotypes for polymorphic alleles (Kb) at loci				
		DXS85	DXS7	DMD	DXS164	DXS269
6	F	7	9		7.5	3.5
	M	14/7	12/9		8.7/8.7	6.8/3.5
	P	14(2)/7(3)	12(2)/9(3)		8.7(4)/7.5	6.8(2)/3.5(3)
7	F	7	12	20; 8	8.7	6.8
	M	14/7	12/12	20/20; 15/15	8.7/7.5	6.8/6.8
	P	14(2)/7(2)	12(4)	20(4); 15(4)	8.7(2)/7.5(2)	6.8(4)
8	F	14	12	20; 8	7.1	6.8
	M	14/14	12/12	20/20; 15/15	9.4/9.4	6.8/6.8
	P	14(4)	12(4)	20(4); 15(4)	9.4(4)	6.8(4)
9	F	7	9	20; 15	1.6	3.5
	M	7/7	12/9	20/20; 15/8	2.8/2.8	3.5/3.5
	P	7(4)	12(2)/9(2)	20(4); 15(2)/8(2)	2.8(4)	3.5(4)
10	F	14	9		7.5	6.8
	M	14/7	12/12		8.7/7.5	6.8/3.5
	P	14(2)/7(2)	12(4)		8.7(2)/7.5(2)	6.8(2)/3.5(2)

Numbers in parentheses are the copy number of fragments. Underlined genotypes are informative.

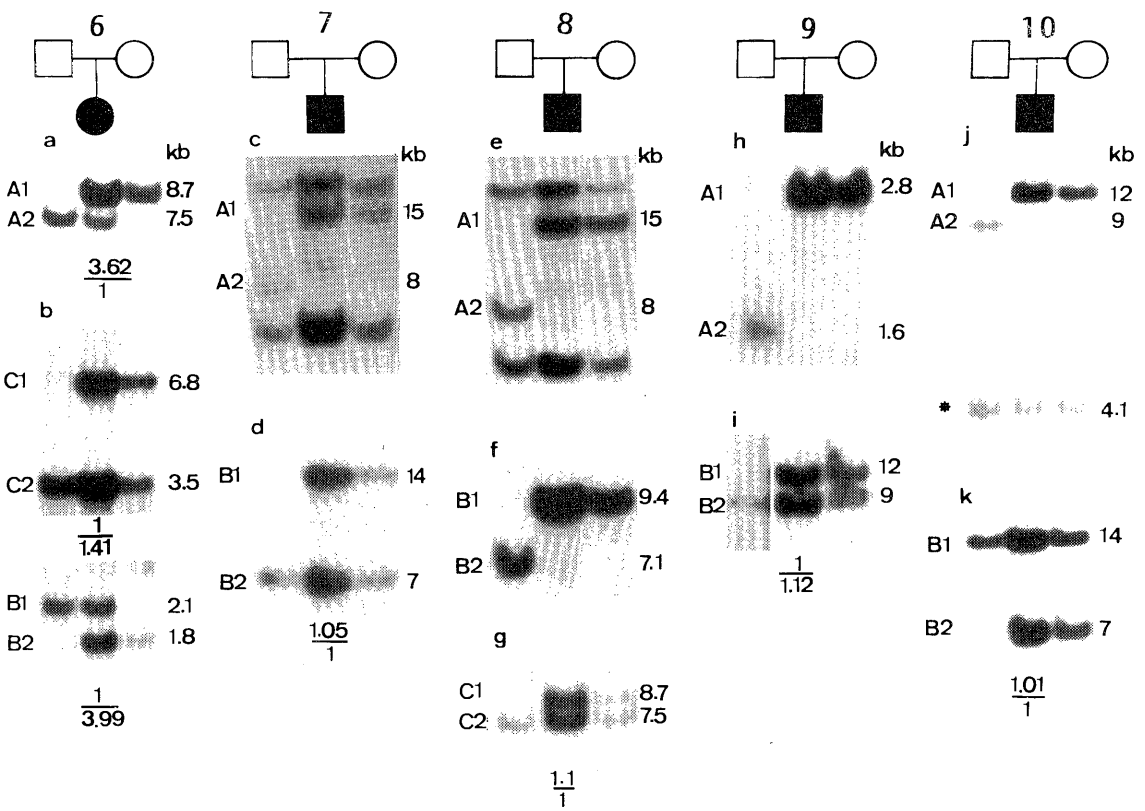

Fig. 2. RFLPs in patients 6-10 with poly-X chromosomes and their parents. A density ratio of one polymorphic fragment (A1-C1) to the other (A2-C2) for each patient is shown at the bottom of the lane. Fragment size is shown in Kb. Probes/enzymes used are pERT87-1/*Xmn*I (a, g), p20/*Msp*I (b), cDMD1a/*Pvu*II (c, e), L782/*Eco*RI (d, k), pERT87-15/*Bam*HI (f), pERT87-15/*Xmn*I (h), L1.28/*Taq* I (i, j), and p20.36/*Taq*I (k, asterisk) for an internal density control.

Table 5. Results of RFLP studies on del(15) and t(15q15q)

Family number	Family member	Copy nember of polymorphic DNA fragments (Kb)			
		DI5S9	D15S11	D15S10	D15S18
11	F	6.5/6.5	1.0/1.0	9.0/9.0	8.5/8.5
	M	6.5/6.5	1.2/1.0	9.0/8.2	14.0/8.5
	P	6.5/-	1.2/-	-/8.2	14.0/8.5
12	F	6.5/6.5	1.2/1.2	9.0/8.2	14.0/14.0
	M	6.3/6.3	1.2/1.2	9.0/8.2	14.0/14.0
	P	-/6.3	1.2/-	-/8.2	14.0/14.0
13	F	6.5/6.5	1.2/1.2	9.0/8.2	8.5/8.5
	M	6.5/6.5	1.2/1.0	9.0/8.2	14.0/8.5
	P	6.5/6.5	1.2/1.2	9.0/-	14.0/8.5
14	F			9.0/8.2	14.0/14.0
	M			9.0/8.2	8.5/8.5
	P			9.0/-	14.0/8.5
15	F	6.5/6.3	2.4/0.9	9.0/9.0	14.0/14.0
	M	6.5/6.5	3.4/2.4	9.0/8.2	14.0/14.0
	P	6.5/6.5	2.4/2.4	9.0/8.2	14.0/14.0
16	F		1.2/1.2	9.0/9.0	
	M		1.2/1.0	9.0/8.2	
	P		1.0/1.0	9.0/9.0	
17	F		3.4/2.4	9.0/8.2	14.0/8.5
	M		3.4/2.4	9.0/8.2	14.0/8.5
	P		2.4/2.4	8.2/8.2	14.0/14.0

Underlined genotypes are informative for the origin.

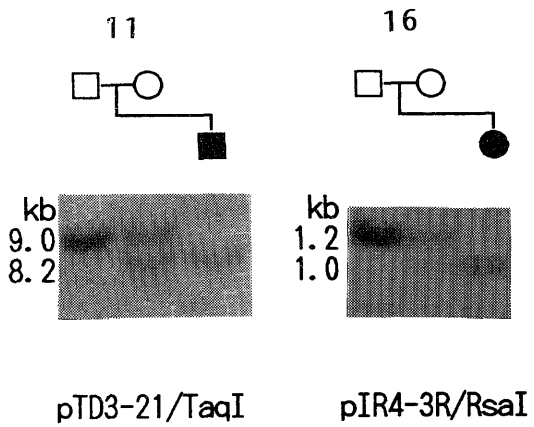

Fig. 3. The pTD3-21/*Taq*I RFLP segregation in family 11 with del (15) (q11.1q12) (a), and pIR4-3R/*Rsa*I RFLP in family 16 with t(15q15q) (b).

7). The result of D15S11(pIR4-3R)/*Rsa*I analysis in this family were also consistent with the paternal origin. Similar results were obtained in family 12 by D15S9(pML34)/*Sca*I analysis, indicating the paternal origin for the deletion in patient 12 (**Tables 5 and 7**). The other 3

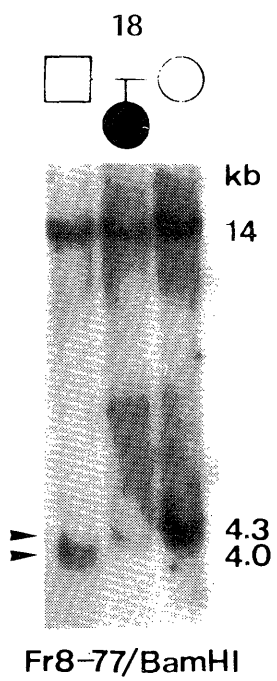

Fig. 4. The Fr8-77/*Bam*HI RFLP in patient 18 with partial monosomy 21 and her parents.

Table 6. Results of RFLP studies on structural X-chromosome abnormalities

Family number	Family member	Genotypes for polymorphic alleles (Kb) at loci								
		DXS85	DXS41	DMD		DXS164	DXS270	DXS84	DXS7	DXS15
19	F			5.8;	8	8.7		-/2.4		
	M			20/20;	15/8	8.7/7.5		4.2/2.4		
	P			20/5.8(2);	15/8(2)	8.7(3)		4.2/2.4(2)		
20	F	14	13			2.7				
	M	14/14	22/22			3.8/3.8				
	P	14/-	22/-			3.8/-				
21	F		22	20;	8	3.8			12	5.8
	M		22/22	20/20;	15/15	3.8/3.8			12/12	5.8/2.8
	P		22/-	20/ ;	8/-	3.8/-			12/-	5.8(3)
22	F	7				7.5			12	
	M	14/14				7.5/7.5			12/12	
	P	-/7				7.5/-			12/-	
23	F	14					21		12	
	M	14/7					16/16		12/9	
	P	14/-					21/-		12/-	
24	F					8.7	16		12	
	M					8.7/7.5	16/16		12/9	
	P					8.7/7.5(2)	16(3)		12(3)	
25	F	7				8.7			12	
	M	7/7				8.7/7.5			12/9	
	P	7/-				-/7.5			-/9	

The number in parentheses are the copy number of fragments. Underlined genotypes are informative.

families (families 13-15) were all uninformative.

15q; 15q translocation: In one (family 16) of the two families, the origin and the mechanism of formation could be traced (**Tables 5 and 7**). The genotypes for the D15S11(pIR4-3R)/*Rsa*I fragments in the father, the mother and the patient (patient 16) were 1.2kb/1.2kb, 1.2kb/1.0kb, and 1.0kb/1.0kb, respectively (**Table 5, Fig. 3b**), indicating that both arms of the 15; 15 translocation chromosome in the patient was derived from a maternal chromosome 15. The mechanism of formation was deduced as a centric misdivision of a maternal chromosome 15 followed by duplication of its long-arm (**Table 7**). RFLPs detected in the other t(15q15q) patient (patient 17) were not informative.

Partial monosomy 21: A combination of D21S82 (Fr8-77)/*Bam*HI gave a 4.3kb/4.0kb RFLP. In family 18, analysis with this combination showed that the father was a 4.0kb/4.0kb homozygote, the mother a 4.3kb/4.3kb homozygote, and the patient 18 a 4.3kb/- hemizygote (**Table 3, Fig. 4**). This observation leads to the conclusion that breaks occurred between paternal chromosomes 2 and 21 during spermatogenesis, and both the 21pter-21q21.3 and the 2q37.3-3qter segments were lost after the reunions of the chromosomes (**Table 7**).

Structural X chromosome abnormalities: The origin was ascertained in all of the seven patients (patients 19-25) (**Tables 6 and 7**). In family 19, a combination of DMD(cDMD1a)/*Pvu* II gave two pairs of polymorphic alleles (20kb/5.8kb, and 15kb/8kb)[3]. The father was hemizygous both for the 8kb and for the 5.8kb alleles, and the mother 15kb/8kb heterozygous and 20kb/20kb homozygous. The patient (patient 19) with dup(Xp) seemed double-heterozygous for the 20kb/5.8kb and for the 15kb/5.8kb alleles. Densitometric analysis revealed that the patient had three copies of the 6.4kb constant fragment, two copies of the 8kb and the 5.8kb polymorphic fragments, and one copy of each of the 20kb and 15kb fragments (**Table 6, Fig. 5a**), indicating that her 5.8kb allele had come from her father in duplicate. This finding was confirmed by the DXS84(754-11)/*Eco*RI RFLPs (**Table 6, Fig.**

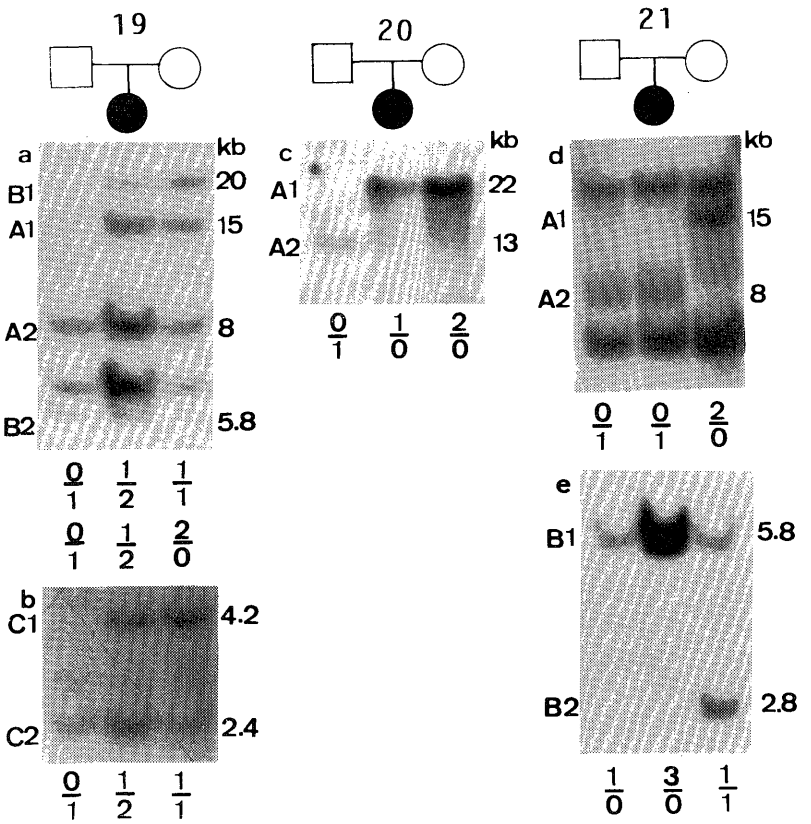

Fig. 5. RFLPs in patients 19-21 with X chromosomal structural abnormalities and their parents. Southern blots with cDMD1a/*Pvu*II (a, d), 754-11/*Eco*RI (b), p99-6/*Pst*I (c), and DX13/*Bgl*II (e). Copy number of alleles (1 and 2) of polymorphic DNA fragments in different loci (A-C) in each family member is shown at the bottom of each lane. Fragment size is shown in Kb.

5b). These findings deduced a mechanism of formation of the dup(Xp) in this case as an unequal sister chromatid exhange in the paternal X chromosome (**Table 7**).

In family 20, a combination of DXS41(p99-6)/*Pst*I indicated that the father was hemizygous for the 13kb allele, the mother homozygous for the 22kb allele and the patient, with del(X)(p11) (patient 20), hemizygous for the 22kb allele (**Table 6, Fig. 5c**). The result indicated that in the patient an allele that should have been transmitted from her father is missing. Thus, the interstitial deletion in this patient resulted from the loss of chromosomal segment during a breakage-reunion event occurring in a paternal X chromosome (**Table 7**).

Analysis with DMD(cDMD1a)/*Pvu*II in family 21 showed that the father's genotype was 8kb, the mother's genotype 15kb/15kb, and the genotype of the patient with i(Xq) (patient 21) 8kb (**Table 6, Fig. 5d**). Among the 5 probes located on Xq, only the DXS15(DX13)/*Bgl*II combination gave an informative RFLP in this family, showing that the genotypes of the father, mother and the patient were 5.8kb, 5.8kb/2.8kb, and 5.8kb/5.8kb/5.8kb, respectively (**Table 6, Fig. 5e**). These results were consistent with the cytogenetic finding that the patient has one short-arm and three long-arms of X chromosome. Also indicated in the patient were the lack of an allele in the short-arm that should have been inherited from the mother, and

Table 7. Origin and mechanism of formation in 25 cases of chromosome abnormalities

Patient number	Parental origin		Mechanism of formation
	Pateral	Maternal	
1, 3		yes	Nondisjunction at Mat II
2	?	?	Excluding nondisjunction at both Pat I and Mat II
4	?	?	Excluding nondisjunction at Pat I
5			Somatic nondisjunction of paternally derived chromosome 21
6-10		yes	One nondisjunction at Mat I, two nondisjunctions at Mat II
11-12	yes		Break-reunion event
13-15	?	?	Break-reunion event
16		yes	Centric fission and duplication of a maternal chromosome 15
17	?	?	Translocation or centric misdivision
18	yes		Translocation between paternal chromosomes 2 and 21 during paternal gametogenesis
19	yes		Unequal sister chromatid exchange of the paternal X chromosome
20	yes		Break-reunion event or unequal sister chromatid exchange of the paternal X chromosome
21-23		yes	Centric misdivision of a maternal X chromosome
24		yes	Sister chromatid break-reunion of a maternal X chromosome
25	yes		Break-reunion event

?, uninformative ; Mat I, maternal first meiosis ; Mat II, maternal second meiosis ; Pat I, paternal first meiosis

duplication of a maternally-derived allele on the long-arm. Thus i(X) in this patient arose through a centric fission of a matenal X chromosome followed by duplication of its long-arm (**Table 7**). RFLP analysis on the other two patients (patients 22 and 23) with i(Xq) showed the origin and the mechanism of formation similar to those in patient 21 (**Tables 6 and 7**).

In family 24, analysis with DXS164(pERT87-1)/*Xmn*I revealed that the genotypes of the father, mother and the patient (patient 25) were 8.7kb, 8.7kb/7.5kb, and 8.7kb/7.5kb/7.5kb, respectively (**Table 6**). The DXS164(pERT87-15/*Bam*HI combination showed that their genotypes were 7.1kb, 9.4kb/7.1kb, and 9.4kb/9.4kb/7.1kb, respectively. These results indicated that the patient with inv dup(Xq) had inherited two copies of an identical maternal allele and one copy of the paternal allele. Thus, the i dic(Xq) arose through an unequal sister chromatid exchange in a maternal X chromosome (**Table 7**).

In family 25, analysis with DXS7(L1.28)/*Taq*I showed that the genotypes of the father, mother and the patient (patient 26) were 12kb, 12kb/9kb, and -/9kb, respectively (**Table 6**). A long exposed autoradiogram showed a faint 12kb fragment in the patient. Thus, the ring chromosome X in this patient arose at paternal meiosis and became as the primary cell line, followed by the loss of the r(X) at an early mitotic division leading to the 45, X cell line (**Table 7**).

DISCUSSION

The parental origin was ascertained in 8 of the 10 numerical abnormalities analyzed, and in the structural abnormalities, that could be traced in 11 of the 15 cases (**Table 7**). Two of the three structural abnormalities whose origin was unsuccessfully traced were del(15)(q11.1q12).

Since the ascertainment rate depends on the number of available probes and detectable RFLPs, the failure of ascertainment in these cases was due both to the minute deleted segment and to few RFLPs at 15q11.2[16]. In general, the origin study for partial monosomy or trisomy is restricted; we first have to find RFLPs within the chromosomal segment involved in the abnormalities.

The observations on the origin of numerical abnormalities indicate that the parental origin of numerical abnormalities is not different between autosomes and X chromosome, the maternal origin being predominant. In trisomy 21, all of the 3 informative cases were maternal in origin. This biased origin is not inconsistent with the previous cytogenetic data on the origin of an additional chromosome 21 in Down syndrome where a ratio of the maternal to the paternal origin is 4 : 1[15]. The origin of the additional X chromosomes in our 5 poly-X cases were all maternal and the mechanism of formation in all was three nondisjunctions at maternal meiosis. An identical mechanism has been reported by Villamar *et al*[21]. Since the probability of the occurrence of three nondisjunctions in the same ovum seems very rare in general, there must be an underling mechanism (factor) leading to the nondisjunction. Maternal age is thought as one of such factors[19]. However, the average maternal age in the mothers of these 6 patients was 30.2 years, not statistically higher than the average age among mothers in the general population.

The detected origin of the autosomal structural abnormalities were all paternal (**Table 7**). This observation is consistent with the hypothesis by Chamberlin and Magenis[2] that structural chromosomal abnormalities in man is preferentially paternal in origin. An exception may be the deletion of the 15q11.2 segment frequently seen in Angelman syndrome patients where the deleted chromosome 15 is exceptionally maternal in origin[11]. For the occurrence of this syndrome, "paternal genomic imprinting" is presumed as an underlying mechanism. In this context, the result for one t(15q15q) case (patient 16), who suffers from Prader-Willi syndrome, is interesting. Both of the arms of the translocated chromosome in this patient came from one of the maternal homologues 15. This condition is equivalent to "maternal uniparental isodisomy" that is occasionally found in karyotypically normal patients with Prader-Willi syndrome[16]. Since "maternal genomic imprinting" has been proposed as the mechanism responsible for the occurrence of the syndrome, the disease in our patient is likely due to the lack of a paternally derived locus.

The paternal origin of *de novo* partial monosomy 21 [45,XX,-2,-21, +der(2)t(2;21) (q37.3; q21.3)] in patient 18 was ascertained using several 21-linked DNA segments as probes. Among these, the locus of D21S82 (Fr8-77) has not regionally been mapped[7]. The result showing a one-copy density for this DNA in patient 18 in turn indicates the locus of this DNA segment between 21cen21q21.3[1].

The origin of dup(Xp), del(Xp), and r(X) are of paternal origin, while that of i(Xq) in three cases and i dic(Xq) are of maternal origin (**Table 7**). These results, together with those of autosomal structural abnormalities and with autosomal and X chromosomal numerical abnormalities, suggest that the *de novo* abnormalities due to events involving centromere disruption arise predominantly during oogenesis, while those due to simple breakage-reunion events occur preferentially during spermatogenesis. It is most likely that the occurrence of chromosomal aberrations in man is primarily determined by biophysical differences between the female and the male. Females are born with all oocytes arrested in the dictyotene stage (a last stage ofthe first meiosis) and the first meiosis is induced to terminate by gonadotropin stimulation after puberty. It is plausible that during this arresting period, some apparatuses involving cell divisions are functionally disturbed, predisposing to nondisjunction. Thus, the higher female age, the higher risk of nondisjunction is expected. On the other hand, males begin sperm production at puberty undergoing continued proliferation through the adult life. The testis, situated outside the abdomen, is largely unprotected against environmental changes, and turn-over rate of its cells is high. The chromosomes of its cells are thus expected to be susceptible to environmental

damages (breakages), such as those due to temperature changes and radiation. Therefore, most structural abnormalities are of paternal origin.

ACKNOWLEDGEMENTS

I express my gratitude to Professor N. Niikawa, Department of Human Genetics, Nagasaki University School of Medicine, and to Professor J-H Xia, Department of Medical Genetics, National Center of Medical Genetics, Hunan Medical University, China, for their scientific suggestions and reviewing the manuscript. I also thank Drs. Y. Fukushima, I. Kondo, H. Oh-hashi, and many other doctors, for providing blood materials.

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